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Small plasmids carrying *vga(A)* or *vga(C)* genes mediate resistance to lincosamides, pleuromutilins and streptogramin A antibiotics in methicillin-resistant *Staphylococcus aureus* ST398 from swine

Kristina Kadlec¹, Constança F. Pomba², Natacha Couto² and Stefan Schwarz^{1*}

¹Institute of Farm Animal Genetics, Friedrich-Loeffler-Institut, Neustadt-Mariensee, Germany; ²CIISA, Faculty of Veterinary Medicine, Universidade Técnica de Lisboa, Lisboa, Portugal

*Corresponding author. Tel: +49-5034-871-241;
Fax: +49-5034-871-246; E-mail: stefan.schwarz@fli.bund.de

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Sir,
Methicillin-resistant *Staphylococcus aureus* (MRSA) isolates of the sequence type ST398 have been identified recently from cases of exudative epidermitis in swine, but also as colonizers of apparently healthy swine in Portugal.^{1,2} A considerable number of these isolates displayed an unusual resistance phenotype, namely resistance to the lincosamide clindamycin, but susceptibility to the macrolide erythromycin.^{1,2} Since the genetic basis of this resistance phenotype is unknown, we selected five representative isolates to identify the respective resistance genes, to determine whether they are transferable and to investigate their genetic environment.

Of the five MRSA ST398 isolates, three (E32, E33 and E49) were obtained from dust samples in three different holdings of breeding pigs during the European Community baseline study, one (E8) was from the nasal swab of an apparently healthy sow and the remaining one (E18) was from a skin lesion of a piglet suffering from exudative epidermitis. PCR screening for the presence of the lincosamide resistance gene *lnu(A)*³ yielded negative results in repeated experiments using plasmid DNA or chromosomal DNA as targets. A full antibiogram of these isolates, as conducted by broth microdilution according to CLSI document M31-A3,⁴ revealed that besides the previously reported resistance to oxacillin, clindamycin and tetracycline,^{1,2} all five isolates displayed high MIC values of ≥ 128 mg/L for the

pleuromutilin tiamulin and ≥ 32 mg/L for the streptogramin A antibiotic virginiamycin M1. Combined resistance to lincosamides, pleuromutilins and streptogramin A antibiotics has recently been described to be associated with the resistance genes *vga(A)*⁵ and *vga(C)*,⁶ both of which are located on plasmids.

Plasmid profiling revealed 1–3 plasmids in each of the five MRSA ST398 isolates. The plasmids were subjected to transformation into the plasmid-free recipient strain *S. aureus* RN4220, with subsequent selection of the transformants on medium containing clindamycin (2 mg/L).⁶ All five isolates yielded transformants that harboured a single plasmid of either 5.3 (E49) or 5.7 kb (E8, E18, E32 and E33). Susceptibility testing confirmed that all transformants displayed resistance or high MICs (in cases where no applicable breakpoints are available to classify an isolate as resistant) to lincosamides, pleuromutilins and streptogramin A antibiotics. Restriction analysis with the enzymes PvuII, DraI, PstI and BglII showed that the 5.3 kb plasmid, designated pCPS49, differed distinctly from the 5.7 kb plasmids, which were indistinguishable based on restriction analysis with the aforementioned endonucleases, but also with HindIII, PstI, ClaI, EcoRI, XhoI and EcoRV. The plasmid obtained from the E32 transformant, designated pCPS32, was chosen as representative of this group of plasmids for further analysis. HindIII fragments of plasmids pCPS32 and pCPS49 were cloned into pBluescript II SK+, and sequenced completely on both strands.

Plasmid pCPS32 was 5718 bp in size (accession no. FN806791). It showed 99.9% sequence identity to the 5713 bp plasmid pVGA obtained from a human clinical *S. aureus* in Portugal.⁵ Sequence analysis revealed the presence of three open reading frames (Figure 1). The first reading frame coded for a Rep protein of 312 amino acids involved in replication of plasmid pCPS32. The second reading frame encoded a Vga(A) ABC transporter protein of 522 amino acids that is responsible for combined resistance to lincosamides, pleuromutilins and streptogramin A antibiotics. The third reading frame coded for a Mob protein of 325 amino acids involved in plasmid mobilization. While the Rep and Vga(A) proteins of pCPS32 were indistinguishable from those of pVGA, the Mob protein showed a single amino acid exchange, Thr50 (pCPS32) versus Ala50 (pVGA).

Plasmid pCPS49 was 5292 bp in size (accession no. FN806792). It also comprised three reading frames for proteins involved in plasmid replication, antimicrobial resistance and mobilization. Plasmid pCPS49 harboured a *vga(C)* gene encoding a 522 amino acid ABC transporter identical to that described in the revised sequence of plasmid pKKS825 (accession no. FN377602).⁶ The Vga(C) protein also conferred combined resistance to lincosamides, pleuromutilins and streptogramin A antibiotics. The *pre/mob* gene for plasmid recombination and

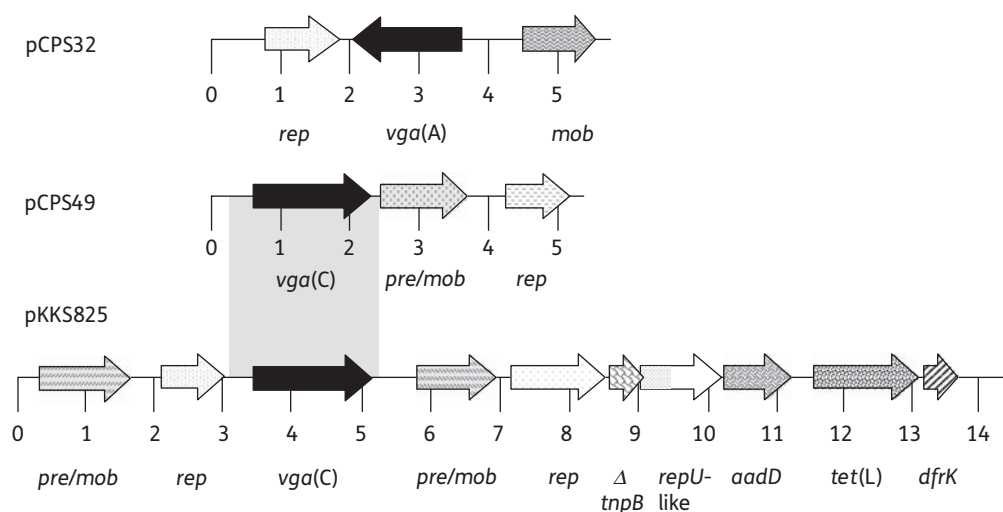


Figure 1. Maps of the plasmids pCPS32 (FN806791) and pCPS49 (FN806792) in comparison with the other reported *vga(C)*-carrying plasmid pKKS825 (FN377602).⁶ The arrows indicate the extents and directions of transcription of the genes *rep*, *repU* (plasmid replication), *tet(L)* (tetracycline resistance), *dfrK* (trimethoprim resistance), *pre/mob*, *mob* (plasmid recombination/mobilization) and Δ *tnpB* (truncated transposase B). The different *rep* and *pre/mob* genes are displayed in different shadings to underline their structural differences. The *vga(A)* and *vga(C)* genes are shown as black arrows. The region of 99.9% homology between pCPS49 and pKKS825 is marked by grey shading. A distance scale in kb is given below each map.

mobilization coded for a 409 amino acid protein, which revealed only minor relatedness to other known Pre/Mob proteins. The best match with sequences deposited in the database were 50.2% and 49.2% identity to 403 amino acid mobilization proteins of *Lactococcus lactis* (YP_001429536) and *Lactobacillus sakei* (YP_001967741), respectively. The 220 amino acid Rep protein of pCPS49 also displayed only 63.9% identity and 62.7% identity to plasmid replication proteins of *L. sakei* (YP_001967739) and *Lactobacillus plantarum* (YP_00133712), respectively. Plasmid pCPS49 showed 99.9% identity to pKKS825 in a 2222 bp segment, which comprised the *vga(C)* gene and 404 bp in the upstream region and 249 bp in the downstream region.

The detection of plasmid-borne *vga(A)* and *vga(C)* genes, which confer a multiresistance phenotype that includes pleuromutilins, in MRSA ST398 isolates from swine is an observation that requires further surveillance. Moreover, pleuromutilins are extensively used either on a prophylactic and/or therapeutic basis in the pig industry in Portugal. The close structural relatedness of the *vga(A)*-carrying plasmid pCPS32 from porcine MRSA with pVGA from human clinical *S. aureus*, both from Portugal, suggests that an exchange of this plasmid type between humans and pigs has already occurred.

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Transparency declarations

None to declare.

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